

Current reviews of allergy and clinical immunology

(Supported by an unrestricted educational grant from Genentech, Inc. and Novartis Pharmaceuticals Corporation)

Series editor: Harold S. Nelson, MD

Anti-IgE as a mast cell-stabilizing therapeutic agent

Tse Wen Chang, PhD,^{a,b} and Yu-Yu Shiung, BSc^a Taipei, Taiwan

This activity is available for CME credit. See page XXA for important information.

After nearly 20 years of development, humanized monoclonal anti-IgE antibodies have been shown in about 20 phase II and III clinical trials to be effective and safe in treating allergic asthma, perennial and seasonal allergic rhinitis, and allergic reactions to peanuts. Omalizumab has been approved in the United States, European Union, and several other countries for treating patients 12 years and older with moderate-to-severe allergic asthma. Although anti-IgE is often referred to as an IgE-neutralizing antibody and can block IgE's binding to high-affinity IgE Fc receptors (FcεRI) on mast cells and basophils, it has multiple immunoregulatory effects. One such effect is that as the result of depleting free IgE, FcεRI on mast cells and basophils is downregulated to less than 5% within a few weeks to a few months of anti-IgE treatment. This renders the mediator-packed inflammatory cells insensitive to allergen stimulation. Hence this therapeutic anti-IgE represents a new class of mast cell-stabilizing agents, reducing FcεRI density on mast cells and basophils and causing them to be insensitive to allergens. This mechanism contrasts with that of cromones, mast cell-stabilizing agents that retard Ca⁺⁺ mobilization and the degranulation process, thus deflating the intracellular activation signal triggered by IgE-FcεRI aggregation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergy, anti-IgE, mast cells, mast cell stabilizing, omalizumab, cromones, FcεRI, sensitivity, receptor density

This article reviews existing therapeutic agents and potential drug candidates that can be used to stabilize mast cells for treating allergic diseases. When mast cells are stabilized, they are not readily triggered by stimulatory factors, such as allergens, to engage in steps leading to the

Abbreviation used
SCF: Stem cell factor

discharge of preformed pharmacologic mediators and the new synthesis of inflammatory lipid mediators and cytokines. Cromones, especially disodium cromoglycate (also referred as cromolyn), which have been used for treating conjunctivitis, allergic rhinitis, and asthma for nearly 30 years, are well known as mast cell-stabilizing agents for their ability to retard Ca⁺⁺ mobilization and the degranulation process.¹⁻³ Several molecules involved in the signaling pathways of mast cells have been proposed as targets for therapeutic intervention. This review compares the effects of cromones and anti-IgE,⁴⁻⁷ a newly developed humanized antibody for treating severe allergic asthma, in stabilizing mast cells.

THE ROLES OF MAST CELLS IN PHYSIOLOGY AND ALLERGY

In developing mast cell-stabilizing therapeutic agents, an immediate question is whether mast cells can be stabilized without compromising their beneficial or protective functions.⁸ Although mast cells have been studied in the past few decades mostly for their adverse roles in causing inflammatory processes that manifest the acute and delayed-phase allergic symptoms, many studies have established their beneficial roles in innate and acquired immunity against bacteria, parasites, and other infectious organisms and in various sentinel and homeostatic functions.⁸⁻¹¹ For example, mast cells play crucial roles in tissue remodeling, tissue repair, angiogenesis, and wound healing in various physiologic or pathologic conditions. Mast cells are found to consist of at least 2 different subclasses based on the differential expression of proteases: those containing tryptase and those containing tryptase and chymase. Studies of mouse mast cells suggest that their phenotypic diversity is much broader than the simple indicators of tryptase and chymase.¹² These various mast

From ^athe Institute of Biotechnology, National Tsing Hua University, and ^bthe Genomics Research Center, Academia Sinica.

Supported by grant no. 94-2320-B007-004 from the National Science Council, Taiwan.

Disclosure of potential conflict of interest: T. W. Chang has consultant arrangements with and owns stock in Tanox, Inc. Y. Y. Shiung has declared that he has no conflict of interest.

Received for publication February 24, 2006; revised April 5, 2006; accepted for publication April 5, 2006.

Reprint requests: Tse Wen Chang, PhD, Genomics Research Center, Academia Sinica, Nankang, Taipei 115, Taiwan. E-mail: twchang@gate.sinica.edu.tw. 0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2006.04.005

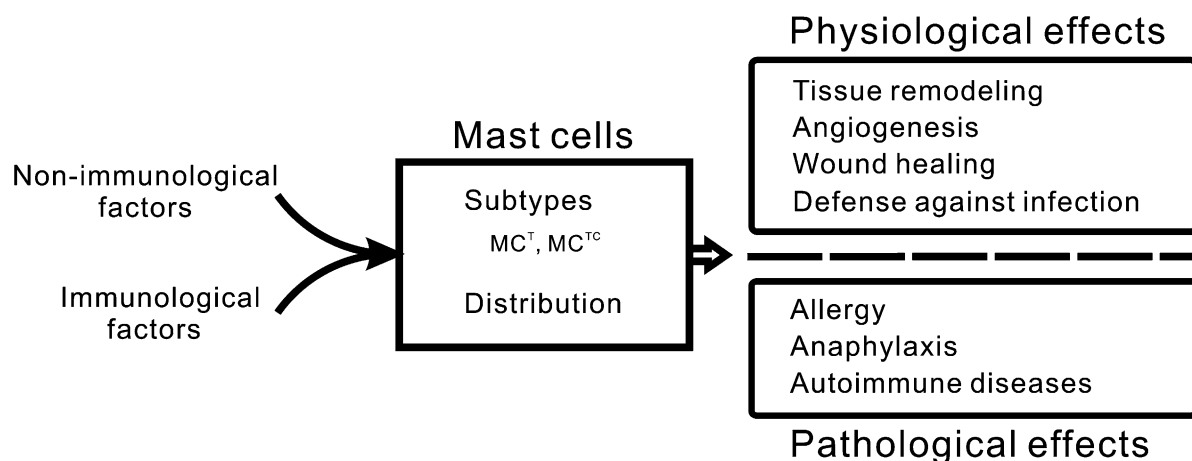


FIG 1. Mast cells play roles in adverse and beneficial functions. Mast cells are found to consist of at least 2 subtypes, those containing tryptase (MC^T) and those containing tryptase and chymase (MC^{TC}), which are distributed in different tissue locations and respond differently to pharmacologic agonists, including immunologic and nonimmunologic stimuli.

cell subtypes respond differently to pharmacologic agonists, including immunologic and nonimmunologic stimuli.^{13,14} Mast cells not only underline the mucosal surface and skin but also copiously line the blood vessels and connective tissues. Mast cells thus play important roles in response not only to foreign innocuous substances but also to infections, autoimmune reactivity, and other physical changes caused by internal and external influences (Fig 1).

It is a plausible rationale that mast cells evolved over millions of years in many animal species for the various beneficial physiologic roles that they play. For these various roles, mast cells are equipped to respond to factors and signals generated in various physiologic conditions, including immune responses to infections and responses to carry out tissue remodeling, tissue development, and wound healing. The mechanistic aspects in the mast cell responses when the mast cells play adverse roles are probably not different from those when they play beneficial roles. However, as people gradually adopt a living environment and lifestyle that drastically differ from those of their ancestors, certain factors that interact with mast cells, such as IgE specific for parasites and for airborne protein substances, are present in our bodies at proportions that are vastly different from those when our immune system evolved.

Mast cells receive numerous stimulatory and inhibitory factors for activation, and some of these factors interact with one another in the signal-transducing pathways, resulting in enhancing or counteracting effects. The responses to allergens, infections, and wounds each produce a different set of factors that can affect mast cells. Type I hypersensitivity reactions are mediated by IgE and factors produced primarily from a T_H2 response.¹⁵ Mast cells and basophils express, on their surface, high-affinity IgE Fc receptors (FcεRI), which are almost entirely bound by IgE. In a sensitized individual the mast cells and basophils are armed because a significant portion of the IgE bound by

FcεRI on these cells is specific for allergens to which the sensitized individual is allergic. Hence on ingesting or inhaling allergens, the incoming allergenic protein molecules can cross-link IgE bound by mast cells, thereby aggregating the underlying FcεRI and triggering the cells to (1) release preformed mediators from granules, (2) synthesize lipid mediators, and (3) activate the genes for many cytokines and chemokines.^{16,17} Mediators of the first 2 classes are mainly responsible for the acute-phase reactions of allergy and those of the third class for the delayed-phase reactions (Fig 2). At the mucosal linings, minute amounts of allergens, which are small, soluble, and often proteolytic proteins,¹⁸ can in some individuals induce IgE responses.

The immune responses to most infections are mediated by factors largely derived from the T_H1 pathway, which results in the production of IgG and various cytokines, such as TNF- α , IFN- γ , IL-2, and others.¹⁹ The response to allergens administered through intracutaneous or intramuscular injections, as performed in desensitization-specific immunotherapy, probably induces a regulatory T-cell or modified T_H2 response.²⁰ Allergen-specific IgG is considered to be a main component responsible for the attenuation effects of specific immunotherapy. Certain subclasses of IgG can also activate mast cells (by binding to Fc γ RIII) in animal models in which the gene for Cε²¹ or for the α chain of FcεRI²² was genetically disrupted. On the other hand, the immune complexes of IgG and allergen can bind to Fc γ RIIB receptors on mast cells, thereby inhibiting IgE and FcεRI-initiated activation pathway,²³ as discussed in more detail in later sections.

FACTORS AFFECTING THE MEDIATOR-CYTOKINE OUTPUT OF MAST CELLS

The output of mediators and cytokines from mast cells in a local tissue or systematically depends on several types

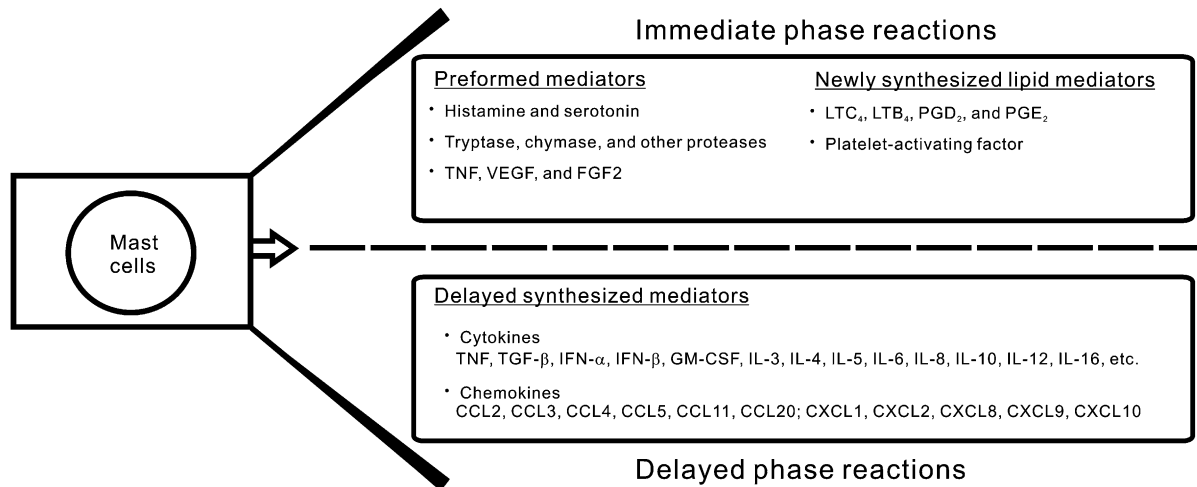


FIG 2. Activated mast cells release and secrete 3 groups of mediators responsible for immediate-phase and delayed-phase reactions. VEGF, Vascular endothelial growth factor; FGF2, fibroblast growth factor 2; LT, leukotriene; PG, prostaglandin.

of factors: (1) the pool size of the mast cells in the local area or in the whole body, where the mast cells will encounter the stimulators and inhibitors that are brought in exogenously or produced in a physiologic or pathologic response; (2) the concentrations and distribution of the stimulators and inhibitors; and (3) the intrinsic properties pertaining to the sensitivity and potency of those mast cells that are to encounter the stimulators and inhibitors (Fig 3).

Pool size and distribution of mast cells

The pool size of mast cells is related to the generation of new mast cells and the survival of existing mast cells. Human mast cells are believed to derive from CD34⁺ hematopoietic progenitor cells in the bone marrow.²⁴ The mast cell-committed precursors circulate as c-kit⁺, FcεRI⁺, and nongranular mononuclear cells,²⁵ which receive additional factors for complete differentiation and maturation into mature granular mast cells. Human mast cells have life spans ranging from several weeks to several months, depending on, among other factors, the tissue locations or organs in which they reside: those lining the skin have a longer life span and those near the mucosal linings of the gut and lung have a shorter life span.^{26,27} Mast cells can be induced to proliferate by a host of factors that act as primary inducers or as cofactors. The major factor inducing the proliferation and maintaining the survival of human mast cells is stem cell factor (SCF),²⁸ which acts through its receptor, c-kit (CD117),²⁹ on mast cells.^{24,25,30,31} IgE can also directly or indirectly maintain the mast cell pool by protecting the cell from apoptosis.³²⁻³⁴ IL-3, IL-4, IL-5, and IL-6 fail to induce mast cell growth but promote their survival.^{35,36} and IL-3, IL-4, and IL-9 act as comitogens when SCF is present.³⁷ In addition, IL-4 has major priming effects on mast cell signaling pathways, IgE receptor expression, and exocytosis.⁸ Thus it is likely that the reactive hyperplasia of mast cells in the

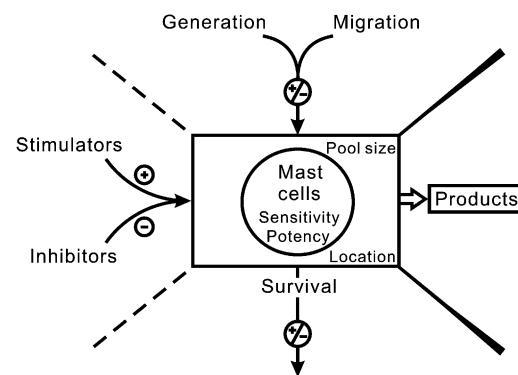


FIG 3. The various influences that affect the outputs of mediators from mast cells. For the generation, maintenance, and survival of mast cells, most factors exert positive effects, whereas a minority of factors exerts negative influences.

mucosal surface that occurs as a feature of allergic inflammation (a mechanism that likely increases the pool size in active mucosal disease) reflects the actions of these cytokines. On the other hand, TGF-β not only inhibits mast cell proliferation but also inhibits SCF to protect mast cells from apoptosis on the removal of IL-3.³⁸

How mast cells traffic into and establish residence in various tissues (mast cells normally do not circulate in the blood) is poorly understood. The local distribution of mast cells is believed to be affected by immunologic responses or inflammatory conditions in the local area, and the products of various cell types affect the recruitment of mast cells into a local area. In particular, mast cells and T cells attract each other, depending on which is activated first, and secrete their array of cytokines and chemokines in a local tissue.³⁹ Many cytokines and chemokines produced by activated T cells and other cell types, such as SCF, IL-8 (CXCL8), TGF-β, RANTES (CCL5), eotaxin

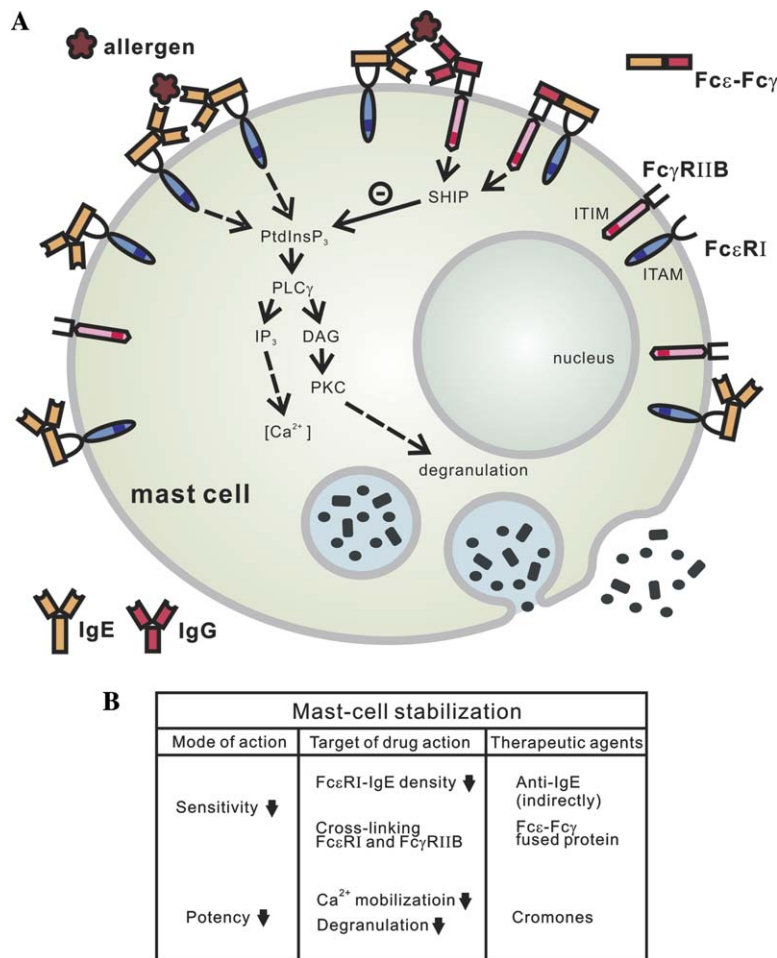


FIG 4. Potential target sites for mast cell-stabilizing agents. **A**, The FcεRI-initiated stimulatory signal-transducing pathway, the FcγRIIB-initiated inhibitory signal-transducing pathway, and the mechanism by which the latter pathway inhibits the former pathway. **B**, The mast cell-stabilizing effects can be classified into those that reduce sensitivity and those that reduce the potency of the mast cell. *SHIP*, Src homology 2 domain-containing inositol 5-phosphatase; *ITIM*, immunoreceptor tyrosine-based inhibitory motif; *ITAM*, immunoreceptor tyrosine-based activating motif; *PLCγ*, phospholipase Cγ; *IP₃*, inositol-1,4,5-triphosphate; *DAG*, diacylglycerol; *PKC*, protein kinase C.

(CCL11), and fractalkine, a membrane-bound chemokine expressed on diverse cell types of the skin, can attract the migration of mast cells.^{40,41} The recruitment of mast cells within the walls of the airway is also affected by products of the airway smooth muscle.⁴² It has been suggested that lung mast cells reside in these tissues (because of a certain microenvironment created by the airway smooth muscle cells), and direct contact between mast cells and smooth muscle cells is important. Furthermore, the pool size of mast cells and the relative proportions of mast cell subtypes containing tryptase and those containing tryptase and chymase in a local area will affect their output of mediators on the stimulation by immunologic and nonimmunologic factors.

Stimulators and inhibitors of mast cells

A large number of substances that stimulate mast cells to swiftly discharge preformed mediator granules and to

synthesize lipid mediators and cytokines have been identified and studied. These substances are of both immunologic and nonimmunologic nature, and the immunologic substances are either IgE related or non-IgE related. Most stimulators bind to surface receptors on mast cells, and others bind to intermediates along the signal-transduction pathways, leading to the release and synthesis of mediators.

The best-studied stimulators are allergens (Fig 4) acting through IgE-bound FcεRI and its linked signal-transducing pathway.⁴³⁻⁴⁶ It should be noted that IgE by itself cannot be considered as a stimulator of mast cells. In fact, because of the high affinity of FcεRI for IgE binding, FcεRI molecules on a mast cell are almost entirely saturated by IgE molecules during the resting period of the mast cell. The binding of multivalent allergen molecules to IgE on a mast cell causes the cross-linking and aggregation IgE-FcεRI. Histamine and peptidoleukotriene,

which are products of mast cells, can also activate or potentiate the activation of mast cells, suggesting a positive-feedback amplification effect of mast cell activation (more details are given in a later section).

A range of substances can stimulate mast cells independently of the IgE-FcεRI pathway. Some allergens, such as the dust mite Der p 1, a serine protease, and bee venom phospholipase A2, can stimulate mast cells without binding to IgE.⁴⁷ Certain neuropeptides (eg, substance P), defensins, and eosinophil products can also stimulate mast cells through their specific receptors and G(i) protein-mediated signal-transducing pathways.⁴⁸

Inhibitors of mast cell activation bind to surface receptors or substrates on mast cells or act on intermediates along the signal-transduction pathways, leading to the generation of molecules that interfere with the signal-transducing processes initiated by the stimulators. Among the better known inhibitory pathways of mast cell activation are those involving the receptors FcγRIIB,²³ gp49B1,⁴⁹ and mast cell function-associated antigen,⁵⁰ all of which contain immunoreceptor tyrosine-based inhibitory motif elements (Fig 4). The natural ligand for FcγRIIB is antigen-IgG complexes, and the natural ligand for gp49B1 is the integrin molecule α_vβ₃ expressed on vesicular endothelial cells. Many other substances, including certain cytokines secreted by various cell types and a large number of natural substances (eg, herbal extracts and compounds), have been identified as possessing inhibitory effects on mast cell activation initiated by immunologic or nonimmunologic stimuli.

ANALYSIS OF THE STABILITY OF MAST CELLS

As discussed above, the total output of mediators and cytokines from mast cells in a tissue location or systemically is a function of the pool size of the mast cells in the local area or in the body and the composition of the stimulators and inhibitors the mast cells receive. In addition, the intrinsic properties of the mast cells in terms of their sensitivity and potency also play important parts in the synthesis and secretion of their mediators and cytokines.

Definition of mast cell stabilization

The term *mast cell stabilization*, which has been used in thousands of articles in the context of mast cell-stabilizing agents, appears to mean “making the mast cell inactive.” However, the word *stabilization* has the connotation that the mast cells in an inflammatory state brought about by an allergic response or by other stimuli are intrinsically unstable. Indeed, in most cases involving mediator release from mast cells, the mast cells merely perform their functions, responding to a stimulation signal brought in externally (eg, allergens) or generated internally (eg, substance P). For example, in an asthma attack induced by contact with a cat, the mast cells lining the bronchial wall release an array of mediators. This is not a result of some intrinsic

and spontaneous excess activity of the mast cells but from their exposure to inhaled cat dander proteins. Thus the element of abnormality is likely that the patient produces allergen-specific IgE in quantities comprising a significant percentage of total IgE. Hence the FcεRI on the mast cells in the patient are bound by the allergen-specific IgE at an abnormally high proportion.

In this article, for the purpose of analyzing the drug actions of therapeutic agents that are being used or developed for attenuating the activities of mast cells, the term *mast cell stabilization* is defined as “making mast cells insensitive or impotent.” Here the sensitivity attribute pertains to the degree of responsiveness of the mast cell to stimulators, more specifically, to the efficiency that the mast cell is triggered to engage its signal-transducing cascade on interaction with the stimuli. The potency attribute pertains to the efficiency that the mast cell releases and synthesizes the mediators once the intracellular activation messenger molecules have been generated.

Sensitivity aspect of mast cells

In a general analysis the sensitivity of a mast cell toward a stimulator appears to be related to the density of the receptor for the stimulator and the competence of the signal-transduction process. For example, the sensitivity of a mast cell toward stimulation by an allergen is related to the IgE-FcεRI density on the surface of the mast cell and the intactness of the signal-transducing pathway initiated by the cross-linking and aggregation of FcεRI. Thus if IgE-FcεRI becomes sparse on the mast cell, it will take a higher concentration of an allergen to induce its cross-linking and aggregation. Alternatively, if the IgE in IgE-FcεRI is bound by an antagonist to block allergen binding, or if the α subunit in FcεRI is bound by an antagonist to block IgE binding, the mast cell will become insensitive to allergen stimulation.

A mast cell can also become less sensitive to a stimulator if the signal initiated by the binding of the stimulator to the receptor at the cell surface cannot be effectively transduced through the pathway because of a certain defective or inhibitory component in the pathway. A defect can be caused by a genetic mutation, interference by an exogenously introduced drug, an intracellular inhibitory intermediate, or other causes. An inhibitor of mast cell sensitization can cause such interference in the stimulatory signal-transduction pathway. For example, the binding of IgG-allergen complexes to the FcγRIIB on the mast cell surface can lead to the production of SH2 domain-containing inositol 5-phosphatase inside the cytoplasm, which interrupts the signal-transduction process initiated by the aggregation of IgE-FcεRI (Fig 4).⁴⁶

Potency aspect of mast cells

The products generated by a stimulator-initiated signal-transduction pathway inside a mast cell will lead to a number of cellular processes, including the discharge of packed and preformed mediators from granules through exocytosis, the *de novo* synthesis of lipid mediators, and the activation of certain genes and the synthesis of their

encoded cytokines and chemokines. The degranulation process requires mobilizing Ca^{++} ions from the Ca^{++} reservoir in the endoplasmic reticulum and involves the actions of several cellular components, including ion channels and cytoskeletons. It is conceivable that the exocytotic and degranulation processes can be inhibited by agents that interrupt one of these cellular components. It is now well established that the synthesis of lipid mediators through arachidonic acid metabolism and the activation of cytokine genes (at least in the case of FcεRI-coupled activation) are coupled with Ca^{++} mobilization.^{43,51}

LONG-USED CROMONES ARE MAST CELL-STABILIZING DRUGS

Probably the best known and studied mast cell-stabilizing drugs are cromones, including disodium cromoglycate and sodium nedocromil,^{1-3,52} which are used primarily as prophylactic treatment for conjunctivitis, early and mild allergic rhinitis, and mild asthma. Cromones have been shown to have effects in improving many types of inflammatory conditions involving various types of tissues. These inflammatory conditions are caused by both immunologic (especially allergic and autoimmune) and nonimmunologic responses. An example of a nonimmunologic response occurs in ischemia-induced cardiac tissue damage in rat models.⁵³

Although cromones have been used for 3 decades, their molecular pharmacologic mechanisms are still not well understood. Nonetheless, cromones are commonly referred to as mast cell-stabilizing drugs. In *in vitro* studies in which mast cells are activated by allergens or polyclonal antibodies specific for IgE (for cross-linking IgE-FcεRI), cromones have been shown to inhibit Ca^{++} mobilization, transport of chloride ions, and the exocytotic process. As a result, the discharge of mediators is inhibited. Because the production of arachidonic acid is coupled with Ca^{++} influx,⁵⁴ cromolyn also inhibits the synthesis of lipid mediators.⁵⁵ The synthesis of lipid mediators generally couples with degranulation.⁸ However, some stimuli induce mast cells to increase cytokine transcription without the degranulation process or lipid mediator production. For example, the stimulation of mast cells by LPS through Toll-like receptor 4 does not involve Ca^{++} .⁵⁶ By contrast, the stimulation of mast cells by cysteinyl leukotrienes involves Ca^{++} .⁵⁷ Furthermore, the drug action of cromones toward their cellular targets is not very specific because they have been shown to inhibit the activation of not only mast cells but also neutrophils, eosinophils, monocytes, alveolar macrophages, and lymphocytes.^{58,59}

ANTI-IGE AS A MAST CELL-STABILIZING AGENT

Anti-IgE is potentially a new treatment option for many allergic diseases

Since the therapeutic approach was conceptualized in 1987,⁶⁰⁻⁶² anti-IgE humanized antibodies, including

omalizumab and TNX901, have been shown in about 20 phase II and III human clinical studies to be safe and efficacious in treating allergic asthma,⁶³⁻⁶⁷ seasonal and perennial allergic rhinitis,⁶⁸⁻⁷⁰ allergic and anaphylactic reactions to peanuts,^{71,72} allergic reactions to latex products,⁷³ and others. It was recently reported that omalizumab pretreatment can decrease acute reactions after rush immunotherapy.⁷⁴ Omalizumab has been approved in the United States, European Union, Australia, Canada, Brazil, and several other countries for treating patients with moderate-to-severe (or severe) asthma. As the allergists gain more experience in using anti-IgE therapy in the future, this new therapeutic modality might be applied more broadly to severe or difficult-to-treat cases of various allergic diseases in which IgE plays a major pathogenic role.

Binding properties of anti-IgE

In discussing the pharmacologic effects of anti-IgE, it is important to first understand the unique binding specificities of these antibodies, which were derived from a rational drug design approach. An ordinary anti-IgE antibody can bind to IgE molecules already bound by FcεRI on basophils and mast cells, cross-linking the IgE and hence causing the aggregation of the underlying FcεRI. It is conceivable that administering an ordinary anti-IgE antibody, even a humanized antibody, could induce severe anaphylaxis in an injected person. However, the therapeutic anti-IgE, which was obtained by serial screening processes among human IgE-specific mAbs, has a set of binding specificities: (1) they bind to human IgE with a very high affinity and not to any other protein in the blood and interstitial fluid; (2) they bind to membrane-bound IgE on B cells; (3) they do not bind to IgE already bound by FcεRI on basophils and mast cells; and (4) they do not bind to the low-affinity IgE Fc receptors (FcεRII or CD23) on B cells, granulocytes, monocytes, platelets, and many other cell types.⁶⁰ One key explanation for the existence of such anti-IgE antibodies is that the binding sites on IgE for FcεRI and FcεRII and the antigenic site for the therapeutic anti-IgE are overlapping or adjacent to one another on the CH3 domain of IgE.⁷

Pharmacologic effects of anti-IgE

The intended pharmacologic purpose of anti-IgE, as it was initially developed, was to reduce IgE concentrations in the blood and tissue and to decrease IgE synthesis by B cells. As human clinical studies were performed, new pharmacologic mechanisms were unraveled. Anti-IgE is often referred to as an IgE-neutralizing antibody. This description simply attributes anti-IgE's value as a chemical blocker of IgE by its ability to prevent IgE from binding to FcεRI. However, anti-IgE has other multiple pharmacologic and immunoregulatory effects.⁷ This article discusses, among those effects, the value of anti-IgE as a mast cell-stabilizing agent because of its ability to downregulate FcεRI and IgE-FcεRI on basophils and mast cells.⁷⁵⁻⁷⁸ Although this effect does not result from a direct interaction between anti-IgE and a mast cell's

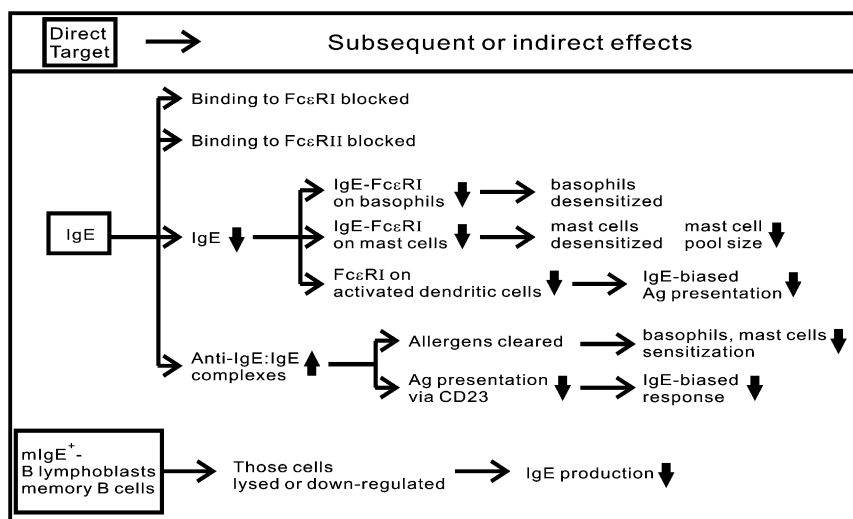


FIG 5. Multiple proved and potential pharmacologic and immunologic effects of anti-IgE. Therapeutic anti-IgE binds to (1) IgE and (2) membrane-bound IgE⁺ B lymphoblasts and memory cells, which each leads to a series of subsequent or indirect effects.

component, it is nevertheless a very important aspect of the pharmacologic effects of anti-IgE.

FcεRI downregulation renders mast cells insensitive

Even before the effects of anti-IgE in downregulating FcεRI on basophils and mast cells were unraveled, it had been reported that the FcεRI density on basophils correlates with serum IgE concentration.⁷⁹ The administration of anti-IgE, which binds to IgE with relatively high affinity (Kd [dissociation constant] in the range of 10^{-10} M), to a patient will reduce free IgE to near zero concentrations in the blood as the subcutaneously injected anti-IgE is dissipated into blood and the extravascular space within a few hours. FcεRI binds to IgE also with comparably high affinity (Kd in the range of 10^{-10} M). The k_{off} (rate constant of dissociation) of IgE-FcεRI is $1.6 \times 10^{-4} \cdot s^{-1}$,⁸⁰ meaning that IgE that is bound by FcεRI will remain bound with a half-life of 10 days.⁸¹ When an FcεRI-bound IgE molecule becomes unbound, it is immediately captured by surrounding anti-IgE molecules because the molar concentration of anti-IgE is much larger than that of FcεRI when a patient is receiving anti-IgE treatment. Consequently, the FcεRI molecules on basophils and mast cells gradually become free of IgE. Those IgE-free FcεRI molecules are not stable and are routed back inside the cells and degraded. Hence the total number of IgE-FcεRI molecules on the surface is gradually decreased on anti-IgE treatment.^{75,82}

In another aspect of IgE-FcεRI dynamics, as new FcεRI molecules are synthesized and displaced on the cell surface because of molecular turnover or cell division, they are not saturated with IgE and are hence unstable and are routed back for degradation. Thus as anti-IgE treatment proceeds, the density of IgE-FcεRI molecules gradually decreases. Basophils have a life span of several

days,^{83,84} and hence replenishing the basophil pool by means of continual cell division results in a loss of FcεRI density. It has been estimated that the density of IgE-FcεRI on basophils decreases to less than 50% in 3 weeks and less than 95% in 3 months.⁷⁶⁻⁷⁸ On the other hand, human mast cells have a longer life span than basophils, ranging from several weeks to several months: those lining the skin live longer, and those lining the mucosal surface of the gut and the respiratory tract live shorter.^{26,27} Nevertheless, as IgE-FcεRI on mast cells undergoes renewal, the newly synthesized FcεRI molecules are not saturated with IgE, and hence the density of IgE-FcεRI also decreases gradually.

When the density of IgE-FcεRI decreases to less than certain physiologic levels, much higher concentrations of allergens are required to sensitize mast cells and to induce a positive reaction in skin prick tests.⁷⁸ It is also conceivable that as the IgE-FcεRI numbers on a mast cell decrease to such sparse levels that preclude their cross-linking by allergens that can only monovalently bind to IgE-FcεRI, even very high concentrations of allergens cannot sensitize the cell.

MAST CELL-STABILIZING EFFECT IS A PART OF ANTI-IGE'S OVERALL EFFECTS

Now that we appreciate that the mast cell-desensitizing effect of anti-IgE is rather dramatic and important in its drug action, should we then refer to or classify anti-IgE as a new class of mast cell-stabilizing agents? To address this question, we must weigh the relative importance and contribution of this mast cell-stabilizing effect in anti-IgE's overall pharmacologic effects. First, anti-IgE has a similar effect on downregulating the IgE-FcεRI density on basophils and desensitizing them, with even faster

kinetics. For an allergic condition or disease, such as the anaphylactic reaction to foods, drugs, and bee venom, which involves basophils systematically, the basophil-desensitizing effect of anti-IgE is probably as important as its mast cell-stabilizing effects.^{71,85} Thus unlike cromones, which are used mostly for mast cell-stabilizing effects locally, anti-IgE can be used to render desensitizing effects on basophils and mast cells systematically.

In clinical trials of anti-IgE in patients with allergic rhinitis and asthma, it was observed that the improvement of allergic symptoms in some patients occurred within 1 to 2 weeks after administering anti-IgE. By this time, the IgE-FcεRI on mast cells has been only marginally down-regulated, and mast cells on the mucosal linings of the upper and lower airway presumably have not yet been desensitized. Thus additional mechanisms must account for the therapeutic effects during the early period of an anti-IgE treatment. One of us (T. W. Chang) has proposed that the IgE-anti-IgE immune complexes, which have accumulated to more than 10 times basal IgE levels within 1 to 2 weeks, probably play important pharmacologic roles in the early phase of an anti-IgE treatment. The immune complexes, although incapable of binding to FcεRI, can bind to incoming allergen molecules, in analogy to allergen-specific IgG, thus sweeping the allergens and preventing them from binding to IgE-FcεRI on mast cells and basophils.

Anti-IgE has several other potential pharmacologic and immunoregulatory effects (Fig 5). It also causes the down-regulation of FcεRI on dendritic cells, notably within 2 weeks of anti-IgE treatment. In addition, unlike IgE-allergen complexes, the tripartite anti-IgE-IgE-allergen complexes can no longer bind to CD23 on B cells to route the allergen through this antigen-presenting pathway. The effect of anti-IgE to alleviate allergic symptoms in asthma, allergic rhinitis, and other allergic diseases might also be partly contributed by the ability of anti-IgE to modulate the chronic inflammatory process by reducing levels of eosinophils and IgE⁺ cells (mast cells), as well as T cells and B cells.⁸⁶ These profound effects help subdue the production of inflammatory cytokines and alleviate the allergic symptoms.⁸⁶ Finally, anti-IgE can bind to membrane-bound IgE-expressing B lymphoblasts and memory cells and potentially inhibit and downregulate those B cells. These various effects might inhibit allergens to induce IgE-favored immune responses and hence might gradually steer a shift in the balance between IgE and non-IgE-related immune pathways.

POTENTIAL TARGETING SITES FOR DEVELOPING OTHER MAST CELL-STABILIZING AGENTS

Pharmacologic mediators of mast cells

A number of articles have reported that several histamine H1-receptor antagonists (eg, olopatadine, desloratadine, pyrilamine maleate, and ketotifen)⁸⁷⁻⁸⁹ and inhibitors of peptidoleukotriene⁹⁰ can stabilize mast cells and decrease their mediator release. It appears that histamine

and peptidoleukotriene released from mast cells can render mast cells more active in releasing mediators, suggesting that the pool of mast cells has a positive-feedback, self-enhancing amplification mechanism of mediator release. The inhibitors of tryptase and chymase can also function as mast cell-stabilizing agents; those for tryptase seem to act both extracellularly and intracellularly, whereas those for chymase act only intracellularly.^{91,92} Thus although the significance of the mast cell-stabilizing effects of those inhibitors needs to be verified *in vivo*, the inhibitors of many mast cell mediators can not only counteract the actions of the mediators but also decrease their release from mast cells.⁹²

Signaling pathways are targets for intervention

The signaling pathways for both stimulators and inhibitors of mast cell activation are potential targets for developing mast cell-stabilizing agents.^{46,93} These pathways contain receptors at the cell surface, their respective associated molecules, and a series of downstream signaling intermediates. The signaling process initiated by inhibitors leads to the production of elements that interfere with the signaling process initiated by the stimulators. For example, the cross-linking of FcγRIIB and FcεRI leads to the production of SH2 domain-containing inositol 5-phosphatase, which inhibits the signaling process initiated by the binding of allergen molecules to IgE-FcεRI. Thus if the pathway led by an inhibitor can be augmented or if the pathway led by a stimulator can be retarded, the mast cells will become less sensitive to stimulators. A proposed therapeutic agent is a chimeric fused protein, consisting of the Fc of the ε chain and the Fc of the γ chain, which can bind and cross-link FcγRIIB and FcεRI, inducing negative signaling in mast cells and basophils.⁹⁴

SUMMARY

The mast cell-stabilizing effects of current drugs for treating allergic diseases are dissected into 2 parts: (1) the reduction of potency or readiness to release and synthesize mediators on intracellular activation signaling triggered by the aggregation of FcεRI and (2) the reduction of sensitivity to extracellular allergen stimulation caused by the loss of IgE-FcεRI on the mast cell surface. The long-used cromones represent the first class of therapeutics, and the new-concept drug anti-IgE represents the second class. The kinetics of FcεRI reduction on the mast cell surface suggests that such effects should be significant after a few weeks of anti-IgE treatment. In the initial few weeks of anti-IgE treatment, additional pharmacologic mechanisms contribute to anti-IgE's therapeutic effects.

REFERENCES

1. Storms W, Kaliner MA. Cromolyn sodium: fitting an old friend into current asthma treatment. *J Asthma* 2005;42:79-89.
2. Edwards AM. The discovery of cromolyn sodium and its effect on research and practice in allergy and immunology. *J Allergy Clin Immunol* 2005;115:885-8.

3. van Cauwenberge P, Bachert C, Passalacqua G, Bousquet J, Canonica GW, Durham SR, et al. Consensus statement on the treatment of allergic rhinitis. *European Academy of Allergology and Clinical Immunology. Allergy* 2000;55:116-34.
4. Lanier BQ. Newer aspects in the treatment of pediatric and adult asthma: monoclonal anti-IgE. *Ann Allergy Asthma Immunol* 2003;90:13-5.
5. Busse W, Neaville W. Anti-immunoglobulin E for the treatment of allergic disease. *Curr Opin Allergy Clin Immunol* 2001;1:105-8.
6. Babu KS, Arshad SH, Holgate ST. Omalizumab, a novel anti-IgE therapy in allergic disorders. *Expert Opin Biol Ther* 2001;1:1049-58.
7. Chang TW. The pharmacological basis of anti-IgE therapy. *Nat Biotechnol* 2000;18:157-62.
8. Boyce JA. Mast cells: beyond IgE. *J Allergy Clin Immunol* 2003;111:24-33.
9. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005;23:749-86.
10. Marshall JS. Mast-cell responses to pathogens. *Nat Rev Immunol* 2004;4:787-99.
11. Stevens RL, Austen KF. Recent advances in the cellular and molecular biology of mast cells. *Immunol Today* 1989;10:381-6.
12. Lunderius C, Xiang Z, Nilsson G, Hellman L. Murine mast cell lines as indicators of early events in mast cell and basophil development. *Eur J Immunol* 2000;30:3396-402.
13. Church MK, Levi-Schaffer F. The human mast cell. *J Allergy Clin Immunol* 1997;99:155-60.
14. Irani AM, Schwartz LB. Human mast cell heterogeneity. *Allergy Proc* 1994;15:303-8.
15. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004;113:395-400.
16. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000;105:847-59.
17. Bingham CO 3rd, Austen KF. Mast-cell responses in the development of asthma. *J Allergy Clin Immunol* 2000;105(suppl):S527-34.
18. Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000;105:1185-93.
19. Szabo SJ, Sullivan BM, Peng SL, Glimcher LH. Molecular mechanisms regulating Th1 immune responses. *Annu Rev Immunol* 2003;21:713-58.
20. Jutel M, Akdis M, Blaser K, Akdis CA. Are regulatory T cells the target of venom immunotherapy? *Curr Opin Allergy Clin Immunol* 2005;5:365-9.
21. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* 1994;370:367-70.
22. Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997;99:901-14.
23. Malbec O, Attal JP, Fridman WH, Daeron M. Negative regulation of mast cell proliferation by Fc gammaRIIB. *Mol Immunol* 2002;38:1295-9.
24. Kirshenbaum AS, Kessler SW, Goff JP, Metcalfe DD. Demonstration of the origin of human mast cells from CD34+ bone marrow progenitor cells. *J Immunol* 1991;146:1410-5.
25. Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: biochemical and functional characterization. *Adv Immunol* 1992;52:333-423.
26. Baghestanian M, Jordan JH, Kiener HP, Bevec D, Agis H, Fritsch G, et al. Activation of human mast cells through stem cell factor receptor (KIT) is associated with expression of bcl-2. *Int Arch Allergy Immunol* 2002;129:228-36.
27. Fukuzumi T, Waki N, Kanakura Y, Nagoshi J, Hirota S, Yoshikawa K, et al. Differences in irradiation susceptibility and turnover between mucosal and connective tissue-type mast cells of mice. *Exp Hematol* 1990;18:843-7.
28. Huang E, Nocka K, Beier DR, Chu TY, Buck J, Lahm HW, et al. The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell* 1990;63:225-33.
29. Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell* 1988;55:185-92.
30. Austen KF, Boyce JA. Mast cell lineage development and phenotypic regulation. *Leuk Res* 2001;25:511-8.
31. Galli SJ, Zsebo KM, Geissler EN. The kit ligand, stem cell factor. *Adv Immunol* 1994;55:1-96.
32. Kitaura J, Song J, Tsai M, Asai K, Maeda-Yamamoto M, Mocsai A, et al. Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the Fc epsilon RI. *Proc Natl Acad Sci U S A* 2003;100:12911-6.
33. Kawakami T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol* 2002;2:773-86.
34. Asai K, Kitaura J, Kawakami Y, Yamagata N, Tsai M, Carbone DP, et al. Regulation of mast cell survival by IgE. *Immunity* 2001;14:791-800.
35. Li L, Krilis SA. Mast-cell growth and differentiation. *Allergy* 1999;54:306-12.
36. Yanagida M, Fukamachi H, Ohgami K, Kuwaki T, Ishii H, Uzunaki H, et al. Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells. *Blood* 1995;86:3705-14.
37. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J Exp Med* 1999;190:267-80.
38. Mekori YA, Metcalfe DD. Transforming growth factor-beta prevents stem cell factor-mediated rescue of mast cells from apoptosis after IL-3 deprivation. *J Immunol* 1994;153:2194-203.
39. Mekori YA, Metcalfe DD. Mast cell-T cell interactions. *J Allergy Clin Immunol* 1999;104:517-23.
40. Olsson N, Taub DD, Nilsson G. Regulation of mast cell migration by T and T cytokines: identification of tumour necrosis factor-alpha and interleukin-4 as mast cell chemotaxins. *Scand J Immunol* 2004;59:267-72.
41. Papadopoulos EJ, Fitzhugh DJ, Tkaczyk C, Gilfillan AM, Sasseti C, Metcalfe DD, et al. Mast cells migrate, but do not degranulate, in response to fractalkine, a membrane-bound chemokine expressed constitutively in diverse cells of the skin. *Eur J Immunol* 2000;30:2355-61.
42. Robinson DS. The role of the mast cell in asthma: induction of airway hyperresponsiveness by interaction with smooth muscle? *J Allergy Clin Immunol* 2004;114:58-65.
43. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilon RI. *Nature* 1999;402:B24-30.
44. Metzger H. The receptor with high affinity for IgE. *Immunol Rev* 1992;125:37-48.
45. Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol* 2003;15:639-46.
46. Ott VL, Cambier JC. Activating and inhibitory signaling in mast cells: new opportunities for therapeutic intervention? *J Allergy Clin Immunol* 2000;106:429-40.
47. Machado DC, Horton D, Harrop R, Peachell PT, Helm BA. Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol* 1996;26:2972-80.
48. Befus AD, Mowat C, Gilchrist M, Hu J, Solomon S, Bateman A. Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action. *J Immunol* 1999;163:947-53.
49. Castells MC, Klickstein LB, Hassani K, Cumplido JA, Lacouture ME, Austen KF, et al. gp49B1-alpha(v)beta3 interaction inhibits antigen-induced mast cell activation. *Nat Immunol* 2001;2:436-42.
50. Abramson J, Xu R, Pecht I. An unusual inhibitory receptor—the mast cell function-associated antigen (MAFA). *Mol Immunol* 2002;38:1307-13.
51. Hirasawa N, Santini F, Beaven MA. Activation of the mitogen-activated protein kinase/cytosolic phospholipase A2 pathway in a rat mast cell line. Indications of different pathways for release of arachidonic acid and secretory granules. *J Immunol* 1995;154:5391-402.
52. Church MK, McGill JL. Human ocular mast cells. *Curr Opin Allergy Clin Immunol* 2002;2:419-22.
53. Parikh V, Singh M. Cardiac mast cell stabilization and cardioprotective effect of ischemic preconditioning in isolated rat heart. *J Cardiovasc Pharmacol* 1998;31:779-85.

54. Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, et al. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 1991;65:1043-51.
55. Nabe T, Yamamoto M, Suga M, Kohno S. Intratracheal dosing with disodium cromoglycate inhibits late asthmatic response by attenuating eicosanoid production in guinea pigs. *Eur J Pharmacol* 2004;497:97-104.
56. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002;109:1351-9.
57. Mellor EA, Austen KF, Boyce JA. Cysteinyl leukotrienes and uridine diphosphate induce cytokine generation by human mast cells through an interleukin 4-regulated pathway that is inhibited by leukotriene receptor antagonists. *J Exp Med* 2002;195:583-92.
58. Kay AB, Walsh GM, Moqbel R, MacDonald AJ, Nagakura T, Carroll MP, et al. Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. *J Allergy Clin Immunol* 1987;80:1-8.
59. Tscipopoulos A, Lassalle P, Joseph M, Tonnel AB, Thorel T, Dessaint JP, et al. Effect of disodium cromoglycate on inflammatory cells bearing the Fc epsilon receptor type II (Fc epsilon RII). *Int J Immunopharmacol* 1988;10:227-36.
60. Davis FM, Gossett LA, Pinkston KL, Liou RS, Sun LK, Kim YW, et al. Can anti-IgE be used to treat allergy? *Springer Semin Immunopathol* 1993;15:51-73.
61. Chang TW, Davis FM, Sun NC, Sun CR, MacGlashan DW Jr, Hamilton RG. Monoclonal antibodies specific for human IgE-producing B cells: a potential therapeutic for IgE-mediated allergic diseases. *Biotechnology* 1990;8:122-6.
62. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, et al. Humanization of an antibody directed against IgE. *J Immunol* 1993;151:2623-32.
63. Lanier BQ, Corren J, Lumry W, Liu J, Fowler-Taylor A, Gupta N. Omalizumab is effective in the long-term control of severe allergic asthma. *Ann Allergy Asthma Immunol* 2003;91:154-9.
64. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184-90.
65. Milgrom H. Anti-IgE therapy in children with asthma. *Minerva Pediatr* 2004;56:469-79.
66. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson GB, Chung KF, et al. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;34:632-8.
67. Berger W, Gupta N, McAlary M, Fowler-Taylor A. Evaluation of long-term safety of the anti-IgE antibody, omalizumab, in children with allergic asthma. *Ann Allergy Asthma Immunol* 2003;91:182-8.
68. Nayak A, Casale T, Miller SD, Condemi J, McAlary M, Fowler-Taylor A, et al. Tolerability of retreatment with omalizumab, a recombinant humanized monoclonal anti-IgE antibody, during a second ragweed pollen season in patients with seasonal allergic rhinitis. *Allergy Asthma Proc* 2003;24:323-9.
69. Kuehr J, Brauburger J, Zielen S, Schauer U, Kamin W, Von Berg A, et al. Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. *J Allergy Clin Immunol* 2002;109:274-80.
70. Adelroth E, Rak S, Haahela T, Aasand G, Rosenhall L, Zetterstrom O, et al. Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;106:253-9.
71. Leung DY, Shanahan WR Jr, Li XM, Sampson HA. New approaches for the treatment of anaphylaxis. *Novartis Found Symp* 2004;257:248-64, 76-85.
72. Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986-93.
73. Leynadier F, Doudou O, Gaouar H, Le Gros V, Bourdeix I, Guyomarch-Cocco L, et al. Effect of omalizumab in health care workers with occupational latex allergy. *J Allergy Clin Immunol* 2004;113:360-1.
74. Casale TB, Busse WW, Kline JN, Ballas ZK, Moss MH, Townley RG, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2006;117:134-40.
75. MacGlashan D Jr, Xia HZ, Schwartz LB, Gong J. IgE-regulated loss, not IgE-regulated synthesis, controls expression of Fc epsilon RI in human basophils. *J Leukoc Biol* 2001;70:207-18.
76. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW Jr. The relationship between serum IgE and surface levels of Fc epsilon R on human leukocytes in various diseases: correlation of expression with Fc epsilon RI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* 2000;106:514-20.
77. MacGlashan D Jr, McKenzie-White J, Chichester K, Bochner BS, Davis FM, Schroeder JT, et al. In vitro regulation of Fc epsilon RIalpha expression on human basophils by IgE antibody. *Blood* 1998;91:1633-43.
78. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
79. Malveaux FJ, Conroy MC, Adkinson NF Jr, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-81.
80. Hashiguchi S, Nakashima T, Nitani A, Yoshihara T, Yoshinaga K, Ito Y, et al. Human Fc epsilon RIalpha-specific human single-chain Fv (scFv) antibody with antagonistic activity toward IgE/Fc epsilon RIalpha-binding. *J Biochem (Tokyo)* 2003;133:43-9.
81. MacGlashan D Jr. IgE and Fc epsilon RI regulation. *Ann N Y Acad Sci* 2005;1050:73-88.
82. Borkowski TA, Jouvin MH, Lin SY, Kinet JP. Minimal requirements for IgE-mediated regulation of surface Fc epsilon RI. *J Immunol* 2001;167:1290-6.
83. Wedemeyer J, Galli SJ. Mast cells and basophils in acquired immunity. *Br Med Bull* 2000;56:936-55.
84. Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 2000;12:624-31.
85. Saini SS, MacGlashan DW Jr, Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, et al. Down-regulation of human basophil IgE and Fc epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J Immunol* 1999;162:5624-30.
86. Holgate ST, Djukanovic R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. *Clin Exp Allergy* 2005;35:408-16.
87. Wang YH, Tache Y, Harris AG, Kreutner W, Daly AF, Wei JY. Desloratadine prevents compound 48/80-induced mast cell degranulation: visualization using a vital fluorescent dye technique. *Allergy* 2005;60:117-24.
88. McGill JJ. A review of the use of olopatadine in allergic conjunctivitis. *Int Ophthalmol* 2004;25:171-9.
89. Nagler A, Segal V, Slavin S, Levi-Schaffer F. Ketotifen therapy in chronic graft-versus-host disease (cGVHD): effect on mast cells and fibroblasts. *Clin Exp Immunol* 1995;100:529-35.
90. Tabuchi Y, Kawarabayashi K, Furuhashi K. Inhibitory effect of DS-4574, a peptidoleukotriene antagonist with mast cell stabilizing action, on compound 48/80-induced gastric mucosal lesions in rats. *Agents Actions* 1994;41:21-4.
91. He S, Aslam A, Gaca MD, He Y, Buckley MG, Hollenberg MD, et al. Inhibitors of tryptase as mast cell-stabilizing agents in the human airways: effects of tryptase and other agonists of proteinase-activated receptor 2 on histamine release. *J Pharmacol Exp Ther* 2004;309:119-26.
92. He S, Gaca MD, McEuen AR, Walls AF. Inhibitors of chymase as mast cell-stabilizing agents: contribution of chymase in the activation of human mast cells. *J Pharmacol Exp Ther* 1999;291:517-23.
93. Blank U, Rivera J. The ins and outs of IgE-dependent mast-cell exocytosis. *Trends Immunol* 2004;25:266-73.
94. Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A. A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat Med* 2005;11:446-9.